EFFECTS OF SUPPLEMENTING METHIONINE HYDROXY ANALOG ON BEEF COW PERFORMANCE, MILK PRODUCTION, AND REPRODUCTION

BY

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THESIS

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ABSTRACT

EFFECTS OF SUPPLEMENTING METHIONINE HYDROXY ANALOG ON BEEF COW PERFORMANCE, MILK PRODUCTION, AND REPRODUCTION

Mature Simmental × Angus cows (214 cows; 635 ± 7.4 kg) were utilized to determine the effects of late gestation and early postpartum supplementation of methionine hydroxy analog (MFP; Novus International, Inc., St. Charles, MO, USA) on cow BW, BCS, milk production, milk composition, reproduction, and calf performance until weaning in a fall-calving, cool-season grazing system. Cows were confirmed pregnant prior to experiment, stratified by BW, cow age and AI sire, and assigned to one of twelve pastures (17 or 18 cows·pasture^{-1}). Pastures were randomly allotted to one of two treatments: control (0.45 kg·cow^{-1}·d^{-1} of wheat mid-based pellets, n = 6) or supplement including MFP (0.45 kg·cow^{-1}·d^{-1} of wheat mid based pellets including 10 g MFP, n = 6). Treatments were fed 23 ± 1 d prior to calving through 72 ± 1 d postpartum. Cows were weighed post-calving, at supplementation end, AI breeding, AI pregnancy check, and the end of trial (192 and 193 ± 1 d postpartum). At 74 d postpartum, a subset of cow-calf pairs were used in a weigh-suckle-weigh to determine milk production and milk samples were taken to determine milk composition (n = 45·treatment^{-1}). A serum P_4 assay was utilized to determine cow cyclicity. After supplementation, all cow-calf pairs were managed as a common group until weaning (191 ± 1 d of age). Cows were bred via AI at 97 ± 1 d postpartum and clean-up bulls were turned out 11 d post-AI for a 55 d breeding season. Cow and calf performance were analyzed using MIXED procedure in SAS (SAS Inst. Inc., Cary, NC) and cow reproductive performance and calf health were analyzed using GLIMMIX procedure. Cow BW and BCS were not different ($P \geq 0.10$) at any time points between treatments. There was no treatment effect ($P \geq 0.62$) on calf birth BW, calf weaning BW (191 ± 1 d of age), or calf ADG.

Calculated 24 h milk production did not differ ($P = 0.76$) nor did milk composition or component production ($P \geq 0.21$). There was no difference ($P = 0.69$) in percentage of cows cycling between treatments. Cow AI conception rate and overall pregnancy rate were not different ($P \geq 0.50$) between treatments. No differences ($P \geq 0.61$) in calf health were observed. Supplementation of MFP during late gestation through estimated peak lactation did not affect cow performance, cow milk production, or calf performance when fall-calving cows grazed cool-season forages.

Key Words: cow-calf, fall-calving, late gestation supplementation, methionine hydroxy analog, milk production, reproduction

To the influential individuals who taught me the valuable lessons outside the classroom

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CHAPTER 1

LITERATURE REVIEW

Introduction

Apart from feed costs, cow-calf operations have two areas of concern that can affect their degree of efficiency and determine their economic returns. Both calf weaning weights and calving interval are of high priority to the U.S. cow-calf producer. Weaning weights often are the measure that determines financial returns for a cow-calf production system. Increased calf weights at time of weaning tend to increase potential profits at time of sale. Record high cattle prices recently in 2014 have stirred cow-calf producers to reach for each additional pound at weaning to increase payouts. To achieve increased weaning weights, calves must be provided with the proper nutrition to allow for growth; milk production has a significant effect on calf weight (Rutledge et al., 1971; Beal et al., 1990). Calving interval and percentage are as equally high a priority to the producer as weaning weights. Shortening the calving interval will in turn shorten the time period between calf crops. Decreasing the span between calf crops will coincide with a shortened timeline between economic returns to help support overall livelihood and cover expenses (Ramsey et al., 2005). Proper nutrition is positively correlated with reproductive success, thus, nutrition deserves a great amount of attention because producers can easily manipulate these inputs in a positive or negative fashion (Hess et al., 2005).

Fall-calving production systems have been adapted in certain regions of the country because of more optimal weather conditions for calving during mid-September through mid-November. In addition, market price at sale time of fall-born calves tends to rise in comparison to prices for marketing spring-born calves at time of sale. The price trends are due to increased

demand and short supply of lightweight calves to place on summer grazing programs (Campbell et al., 2013). Fall-calving cows are typically in greater body condition at calving due to the greater availability of pasture for grazing during the summer months preceding calving. Fall-calving cows tend to hit peak lactation when pasture quality still meets energy requirements for lactation. However, fall-calving systems can face challenges if indeed cool season forage pastures cannot meet the increased nutritional requirements of the cow. During the lactation period following parturition, cow dietary requirements increase and additional supplementation may be required to meet increasing needs.

Supplementation

The majority of cow-calf beef operations operate on a predominantly forage based production system. In past years, due to the increased cost of feed, many cow-calf producers have forgone any additional supplementation. Forages alone are not always suitable to meet the nutrient requirements of the cow due to the forage nutritional content and, or in combination with the increasing requirements of the cow during different stages of production. Additional nutrient supplementation may be implemented to assist in addressing deficits that arise from solely grazing forages. Fall-calving herds have the potential to experience more nutritional hurdles following the calving season than spring-calving herds. Warm-season forages tend to decline in quality during the fall-calving season and the cool season forages have not yet produced adequate growth for the increased nutritional demands of the herd during lactation.

In a review, Randel (1990) summarized the implications of undernutrition of energy and protein, both during late gestation and the postpartum period. It is to be expected that weight loss and decreased body fat would be observed if dietary restrictions were experienced during late prepartum periods through postpartum. Cows in a negative energy balance tend to have a longer

period between calving and first postpartum estrus. However, only supplementing cows postpartum may not have the same impact of supplementing cows during both late gestation and early lactation. Inadequate energy intake during late pregnancy lowers fertility rates even when energy intake is adequate during the post calving period. When protein was limited at varying energy intakes during gestation, cows tended to experience lower reproductive performance after calving than herd mates receiving adequate levels of protein across multiple studies (Randal, 1990). Sasser et al. (1988) fed isocaloric diets differing in protein inclusion, cows receiving low protein diets had a lower pregnancy rate (32%) compared to the pregnancy rate of cows fed a high protein diet (74%). Supplementing fall-calving herds during late gestation and immediately following calving may help to address any nutritional deficiencies from grazing late season forages and aid in recovering herd performance. The upfront costs of supplementing late gestating females to meet requirements are negated by the lost returns that could be experienced due to decreased reproductive performance at breeding time. Decreased reproductive performance would affect the succeeding calf crop and overall lifetime profitability of the dam (Patterson et al., 2003).

Limiting Amino Acids

When addressing which key nutrients a supplement should include, it is important to consider what deficiencies become most prominent in cow herds during late gestation and early lactation. Methionine, along with lysine are the two most limiting amino acids in lactating dairy cows (Schwab, 2003; NRC, 2001). Interestingly, gestating dairy cows also have increased requirements for methionine to reach a positive net splanchnic flux (Bach et al., 2000). When the majority of AA supplied to the small intestine are sourced from rumen microbial protein and UIP in the form of metabolizable protein, methionine is the first limiting AA (Waterman et al., 2007;

Richardson and Hatfield, 1978). Grazing cattle obtain the majority of their protein to meet requirements from rumen microbial protein since forages tend to be lower in crude protein than requirements. Additional supplementation may need to be a consideration if AA requirements are not being met.

During late gestation, when the fetus is rapidly growing, there is an increase in the rate of protein accretion and muscle fiber hypertrophy (Du et al., 2010). The increase in protein accretion also warrants an increasing need for metabolizable essential AA, the building blocks of proteins to be supplied by the dam (NRC, 2001). Waterman et al. (2007) hypothesized that post ruminal supplementation of methionine would improve protein accretion of gestating cows, keeping in mind that methionine is the first limiting AA when cattle are predominantly fed forage sources. In a metabolism study using cannulated, late-gestation beef cows, the effects of post ruminal infusion of DL-methionine on N balance and plasma AA concentrations were determined. Basal diets consisted of wheat straw (67% DM), alfalfa hay (32.6% DM) and urea (0.4% DM) and five treatments were tested in the experiment using a replicated 5 x 5 Latin square. Treatments included a control or no urea (NU), urea (U; 0.053 ± 0.002 $g \cdot kg^{-1}$ of BW daily), U + 5 g of $Met \cdot d^{-1}$ (5MU), U + 10 g $Met \cdot d^{-1}$ (10MU), and U + 15 g of $Met \cdot d^{-1}$ (15MU). The treatments were administered twice daily and positioned to allow for direct delivery into the abomasum (Waterman et al., 2007). Excreta samples were taken and analyzed for Kjeldahl N. Blood was collected after the abomasal infusion, and the serum was analyzed for urea and NEFA while plasma was analyzed for concentrations of IGF-1 by RIA.

Waterman et al. (2007) concluded that fecal and urinary N excretion were not affected by urea supplementation, but N excretion decreased linearly in response to methionine infusion. Authors expected that nitrogen retention would increase linearly with the addition of methionine

infusion. Nitrogen retention was improved by 33, 53, 55, and 62 % for cows receiving U, 5MU, 10 MU, and 15 MU, respectively, when compared to the control treatment (Waterman et al., 2007). Increasing methionine supplementation directly to the abomasum showed greatest improvement with the initial 5 $g \cdot d^{-1}$ increase. The increase in N retention demonstrates that late-gestation cows' methionine requirement is not met by urea alone.

Strategic supplementation during late-gestation in fall-calving herds helps to safeguard against nutrient restriction for both the dam and developing fetus. During the last third of gestation, fetal growth rate increases causing a greater demand of nutrients. If this demand is not met, growth performance of the fetus may be impacted due to nutrient restriction (Funston et al., 2010). In a fall-calving herd, the last third of gestation falls in late summer when the nutrient quality and yield of cool-season forages, like tall fescue, may be less than optimal to meet cow production demands due to the "summer slump" in the growth cycle (Roberts et al., 2009). Waterman et al. (2007) suggest that the combination of ruminally undegradable protein (RUP) and microbial protein contributing to metabolizable protein (MP) may not necessarily eliminate certain AA deficiencies. In order to address the deficiency and assist in improving N usage for fetal development and maternal metabolism, Waterman et al. (2007) suggest a strategy to provide a post ruminal supply of the limiting AA. As mentioned, the most limiting AA for lactating cattle is methionine; however, Bach et al. (2000) reported that preparturient cows have increased requirements for methionine as well. Bach (2000) suggests providing 14 $g \cdot cow^{-1} \cdot d^{-1}$ during the last 2 weeks of pregnancy to attain positive net splanchnic flux of methionine.

Modes of Protection

Free AA arise as intermediate products from proteolysis, or the breakdown of proteins in the rumen, and the low concentrations of free AA in the rumen imply that they are rapidly

utilized by rumen bacteria as a nitrogenous nutrient source for growth via deamination, or the removal of the amine group (Chalupa, 1974). For free AA to bypass the rumen to allow for direct uptake by the ruminant animal, supplements would have to be fed in great excess of requirements due to the short half-lives of AA (Chalupa, 1974). Results suggest, from multiple in vitro experiments, there is no justification for free AA supplements in ruminant diets (Kung and Rode, 1996). Feeding of excess free AA can come at a large expense; methods have since been developed to allow for AA to still be supplemented in the diet. Instead of free amino acids, AA are "protected" or capable of "by-passing" the rumen microbial degradation to be absorbed directly by the small intestine to meet the demands of tissues. Treatments such as heating, chemical treatments, altering the solubility, and lipid coating have historically been applied to aid in rumen-stable delivery systems (Wu and Papas, 1997).

Lipid encapsulation is one method of protection allowing for protection in the rumen while still being post-ruminally available. In a study by Smith and Boling (1984), a lipid coating consisting of coconut oil was tested to evaluate if lipid preparation method would be effective in reducing ruminal proteolysis. Cannulated lambs were utilized, testing four diets; a negative control (NC), positive control (PC), additionally two levels of lipid protected methionine, MET-1 meeting methionine requirements, and MET-2 supplying twice the requirement. The protected methionine was delivered as a pellet [mixture of 40% ground corn, 30% coconut oil, 20% DL-methionine, and 10% zein (Smith and Boling, 1984)]. The PC was identical to MET-1; however, the ingredients were delivered individually preventing the coconut oil from directly coating the methionine. Collection of total excreta for nitrogen content was analyzed to determine N digestibility and retention. Blood samples were taken to determine plasma methionine and plasma urea nitrogen.

Results of the Smith and Boling (1984) study determined there were no differences in the apparent dry matter or N digestibilities among diets, which indicates that the coating did not affect digestion. Urinary N tended to be lower when comparing protected methionine diets (MET-1 and MET-2) to the positive and negative control diets (PC and NC), indicating that there was a more efficient utilization of nitrogen on the tissue level. Additionally, the increased efficiency at tissue level of MET-1 and MET-2 resulted in improved N retention by 1.07 g/d (Smith and Boling, 1984). The authors concluded that methionine was limiting at the tissue level and that providing the additional methionine resulted in a positive effect on nitrogen retention. Analysis of blood samples determined plasma urea N was decreased in lambs fed protected methionine diets across post-feeding time points. Post-feeding plasma methionine was increased compared to the negative and positive controls. Plasma analysis indicated that absorption of protected methionine occurred and was transported to the tissue (Smith and Boling, 1984). Results from this study indicated that protection via lipid coating was effective in delivering DL-methionine past the rumen for direct absorption and circulation in the blood stream.

Expense is often associated with the encapsulating of nutrients for livestock feeds as additional processing steps and time are required for the lipid protection method (Emanuele and Putnam, 2006). There are benefits to lipid encapsulating; the improved bioavailability of nutrients to the targeted site of absorption, the protection of specific nutrients during processing steps, and even the increased palatability of ingredients by covering up odors and reducing oxidation of ingredients before feeding. However if rumen bypass of AA can be achieved without the additional cost of lipid ingredients, it could reduce supplement feeding costs.

Methionine Hydroxy Analog

Supplementation of methionine hydroxy analog (MHA) serves as an economical source of rumen-protected methionine compared to L-methionine. Belasco (1972) determined in vitro that MHA was more resistant to degradation in the rumen by rumen bacteria than L-methionine in repeated experiments. In the Exp. 1, 1.5 mg of L-methionine or MHA was anaerobically incubated in rumen fluid to determine products of rumen fermentation in vitro. Exit gasses were collected to measure carbon dioxide ($^{14}CO_2$) and other possible volatile ^{14}C-containing metabolites. Further analysis of in vitro products were used to determine radioactivity and was analyzed using thin layer chromatography (TLC), autoradiography and radioassay of residual ^{14}C-methionine and ^{14}C M-analog. In a preliminary experiment, L-Methionine-1-^{14}C rapidly disappeared, loss of radioactivity amounted to 95%, indicating the free methionine was decarboxylated rapidly, or in other words incorporated into rumen bacterial protein (Belasco, 1972). Comparing ^{14}C-methionine and MHA, ^{14}C-methionine degradation was approximated at 45% decrease in radioactivity over the incubation period. In comparison, there was a 9% loss of the radioactivity associated with MHA over the same incubation. Experiment 2 was a repeat experiment using the same sourced inoculum, 96% of the ^{14}C -Methionine was metabolized after four hours in comparison to 54% of the MHA. Belasco (1972) determined repeatedly that MHA was more resistant to ruminal degradation than methionine. Similarly, Patterson and Kung (1988) reported greater concentrations of remaining MHA (70%) compared to DL-methionine (5%) after 12 h incubation by ruminal microorganisms.

In the same experiment, using calf tissue samples in vitro, Belasco (1972) determined that MHA is capable of being converted to methionine with candidate amino donors by both the liver and kidney with no difference in conversion efficiency between the two tissues. Belasco (1972) used similar procedures to that of Langer (1965) who tested the conversion of methionine

in rat tissues. The calf tissue samples were taken immediately after slaughter. The prepared tissue sample was added to a prepared common substrate which consisted of a calcium salt source of MHA, ^{14}C-M-analog, 1 µM flavin adenine dinucleotide and 10 µM of one of the three nonessential AA supplied as amine donors; glutamine, leucine or asparagine (Langer, 1965). The mixtures were incubated and the supernatants of the reaction were then tested using thin layer chromatography, autoradiography, and radioassay.

Results from the experiment conducted by Belasco (1972) indicated that MHA decreased 25-30% with roughly 12-14% of MHA radioactivity appearing to be converted to methionine with an equal amount of degradation by-products (Belasco, 1972). The minimum total synthesis of methionine in this experiment was 0.60 µM after 4 h incubation. The study also noted the efficiency of the tested nonessential AA as amine donors; glutamine and leucine were equally effective and slightly more efficient than asparagine as amine donors for the conversion of MHA to methionine. Belasco (1972) indicated the nutritional value of MHA for ruminants is due to its resistance to microbial degradation in turn, allowing it to reach the small intestine to be absorbed and utilized by tissues. Additionally the compound's ability to be converted to methionine by both liver and kidney tissues adds to the nutritional efficacy of the compound.

In summary, MHA is thought to bypass rumen bacteria degradation and metabolism because of the chemical structure, allowing it to travel to the abomasum and small intestine to be absorbed directly by the animal. The MHA can then travel to either the liver or kidney where nonessential AA offer an amine group, converting the MHA to L-methionine. The finished product can be used by the animal for the same catabolism functions and requirements as purified L-methionine.

Dosage

Taking into consideration correct dosage of supplemental ingredients is important to ensure performance can be maximized; incorrect concentrations could lead to less than anticipated performance due to over or under supplementing. Under supplementing lactating cows who are deemed methionine deficient would still likely improve performance compared to forgoing any supplementation. However, achieving optimum inclusions would be desired to achieve maximum performance. Over supplementing can be detrimental to profitability in two ways; over supplementing, or feeding in excess, means the animal would be unable to utilize nutrients at the same rate the nutrients were consumed. To combat this issue of over consumption, excess nutrients tend to be excreted by the body, meaning that the additional nutrients were wasted and not cost effective. In some instances when excess nutrients cannot be excreted at the proper rate a toxicity may development. Toxicities may lead to decreases in performance due to decreases in intake or certain instances of tissue damage. Methionine is the most toxic of the essential AA; in mammals if overabundance is prolonged it can cause decreases in voluntary feed intake and tissue damage caused by accelerated cell turnover (Benevenga and Wohlhuter, 1984).

Satter et al. (1974) observed the effects of feeding excess DL-methionine or MHA to determine the toxicity level based on changes in feed consumption of dairy cattle. DL-methionine and MHA treatments were delivered by infusion. Animals were cannulated and received an infusion of one of the two methionine treatments. Feed intakes were measured during the infusion period and significant differences in intakes were reported when depressions in consumption were greater than two standard deviations below the intakes of the experimental animals infused with nontoxic levels of infused methionine.

Satter et al. (1974) concluded ruminal infusion of DL-methionine equivalent to 2.5% of DMI significantly reduced intakes. Interestingly, when the treatment was infused directly into the abomasum, amounts in excess of only 0.6% of DMI resulted in reduced intakes. The surprisingly low level of DL-methionine inclusion to the abomasum suggests that ruminants have greater sensitivity to methionine toxicity (Satter et al., 1974). Both ruminal and abomasal infusions of the MHA treatment reduced DMI at similar inclusion levels and intakes were depressed when MHA was included at 1% on a DM basis. The differences between the toxicity levels between the two treatments in the rumen point to the differences in rumen degradation of the two sources of methionine (Belasco, 1972; Satter et al., 1974). The authors conclude that levels of MHA even nearing 25 $g \cdot cow^{-1} \cdot d^{-1}$ would not be approaching toxicity levels. Majority of supplementation levels in other feeding experiments summarized throughout this review were within the perceived optimum levels for performance.

Cost of Supplementation

There are many variables that contribute to feed costs including but not limited to feed source, processing, delivery method, and additional associated labor. Feed costs have the largest impact on producer profitability in a cow-calf operation (Miller et al., 2001). Delivery of MHA can range from liquid or pelleted supplements, to incorporation into mineral mixes. Cost associated with including 10 $g \cdot cow^{-1} \cdot d^{-1}$ MHA in a supplement feeding program is an additional $\$0.04 \cdot cow^{-1} \cdot d^{-1}$ (Rode, 2014). If management systems already offer daily supplementation or utilize feed delivery then additional cost of MHA may be negligible. However if management systems do not already provide daily supplements, labor costs and feed delivery costs over the duration of the supplementation period may also need to be considered into the total cost of supplementing (Harty and Olson, 2012).

Production and Occurrence of Methionine Hydroxy Analog

Methionine hydroxy analog is a precursor to D,L methionine similar in chemical structure; however, it must undergo certain chemical reactions to be converted into L methionine in vivo (Cooper, 1983). Methionine hydroxy analog differs in chemical structure from D,L methionine at the C_1 carbon in the AA structure; D,L methionine at the C_1 carbon is bound to an amine group (NH_2) while MHA at the C_1 carbon is bound to a hydroxyl group (^{-}OH). The hydroxyl group is then replaced by an amine group by ^{DL}-α-hydroxy acid dehydrogenase via transamination using glutamine or asparagine during the reaction to produce D,L methionine through an energy-dependent process (Cooper, 1983). Different strains of bacteria have been reported to have the capability of converting methionine to MHA. Akobe (1936) found both *Oidium lactis* (fungus) and *Bacillus subtilis* (bacteria) strains had the ability to act on D,L methionine. Belasco (1978) summarized a study in his report referencing Ballio (1961), describing that the fungus strain *Penicillium chrysogenum* when in medium containing methionine could produce the MHA form. Additionally, Maw and Coyne (1966) reported a yeast strain *Saccharomyces cerevisiae* while in a glucose medium also produces MHA when methionine served as the source of sulfur. This early work supports the concept that MHA is a naturally occurring form of methionine with various pathways of formation.

Belasco et al. (1978) conducted an experiment to measure different bacterial and yeast strains' ability to convert ^{L}-[1-^{14}C] methionine to MHA and the natural occurrence of MHA in common food and feed sources. Belasco et al. (1978) tested *Bacillus subtilis* (bacteria), *Saccharomyces cerevisiae* (yeast), *Lactobacillus lactis* (bacteria), and *Lactobacillus bulgaricus* (bacteria) in a sterile skim milk medium to evaluate the conversion ability of methionine to MHA among strains. Thin-Layer Chromatography and GLC were used to separate the

components of the samples and were analyzed using Mass Spectrometry. To determine the natural occurrence of MHA in commercial food, cultured milk products were used. Commercial products included cottage cheese, sour cream, and buttermilk sourced from various U.S. locations; mass spectrometry confirmed MHA was a naturally occurring component in all the milk products (Belasco et al., 1978). When the bacterial strains were compared, *Saccharomyces cerevisiae* was the most efficient in converting methionine to MHA in a 24 h period. *Saccharomyces cerevisiae* converted 19.3% methionine to MHA compared to 4.6% incubated with *B. subtilis* and 5.0% with *L. bulgaricus*. However, it was noted, all strains were capable of converting methionine effectively over a 72 h period. Belasco (1978) noted in his discussion the natural presence of MHA in products other than dairy such as bread, beer, corn silage, DDGS, and sauerkraut. Implications of the study point to the natural occurrence of MHA through bacterial conversion in common foods and feed ingredients. Additionally, both bacteria and yeast strains will readily continue to convert available methionine over time.

Cow Performance

Reproductive Performance

Reducing the calving interval can have a positive effect on profitability. Successful and efficient production systems strive to achieve shorter calving seasons allowing for the greater time for recovery prior to rebreeding. Achieving a shortened duration between calving and cows' first estrus following parturition may require additional nutrition supplementation. Ensuring cows do not fall short of their nutritional requirements while nursing their calf helps to ensure cows do not utilize additional body energy reserves. Supplementation prior to calving through the subsequent breeding season can aid in ensuring nutritional requirements are being met during this critical span. A lengthened anestrous period following calving may decrease a cow's

production potential to less than one calf per year which is considered under average industry standards (Wettemann et al., 2003).

Previous research in dairy cattle has indicated that methionine is the first limiting AA for growing cattle and lactating dairy cows (Chalupa, 1975; Schwab, 2003). Conclusions from these studies would lead us to believe that supplementing a form of bioavailable methionine to lactating beef cattle would be of benefit to meet AA requirements allowing the cow to perform at her highest production potential and achieve subsequent pregnancy during lactation. Following parturition, circulating leutilizing hormone (LH) is low, as time progresses the frequency and amplitude of LH pulses increase approaching the first postpartum estrus (Arije et al., 1974). Improved management techniques have been used to induce the onset of first estrus by administering GnRH through different treatment protocols. Luteinizing hormone released from the pituitary gland is affected by estradiol (E2), consequently nutrient intake and nutrient status influences the response to both GnRH and E2 (Nolan et. al., 1988). Thus, research indicates nutritional status has an influence on LH pulses, or the anovulation period following calving.

In a study to determine effects of restricting CP intake on postpartum LH, late gestation cows were fed one of four treatment diets: adequate crude protein (ADQ; 0.96 $kg \cdot d^{-1}$), deficient crude protein (DEF; 0.32 $kg \cdot d^{-1}$) beginning ninety days (DEF90), sixty days (DEF60), or thirty days (DEF30) prior to estimated calving. Following parturition, cows were maintained on the same treatment diets (Nolan et al., 1988). Blood samples were collected day 20, 40, and 60 over a 6 h period after administration of GnRH to determine circulating LH. From day 20 to day 60, blood samples were also taken to determine progesterone serum levels. Dietary CP and time postpartum affected the response of exogenous GnRH and height of the GnRH-induced LH peak was affected by CP and days after parturition. However, no interaction between CP and days in

the postpartum period was determined. The ADQ cows had a greater magnitude LH peak compared to all DEF cows, and as time postpartum increased, the LH peak also increased linearly in ADQ cows. However, the same pattern for increased LH peak was not observed in DEF cows (Nolan et al., 1988).

The time from GnRH injection to LH peak was significantly affected by CP and tended to be affected by postpartum time. Total amount of LH released after injection was also significantly affected by CP and time postpartum. The ADQ cows' LH response curve was greater than that of DEF cows as well. Overall, results from Nolan et al. (1988) suggest that adequate protein compared to deficient levels of crude protein during pre- and post-partum periods affected LH pulse frequency as time postpartum increased. The increased LH pulses seem to suggest that ADQ cows were closer to estrus in comparison to DEF cows, this is important because a faster return of cyclicity will offer additional breeding opportunities for the following calf crop. The results would indicate that maintaining adequate protein requirements prior to and following calving would elicit a larger LH response to GnRH, leading to a more rapid return to cyclicity.

Clanton and England (1980) conducted three separate experiments on spring calving beef cows; cows grazed range and were supplemented MHA at different inclusion rates from post calving to breeding. The cow performance parameters measured were cow BW changes, onset of estrus, and conception rates. Prior to calving cows were fed hay and following calving, cows were assigned to one of six corn-based supplement treatments with varying levels of MHA inclusion (0, 2, 4, 6, 8, or 10 $g \cdot cow^{-1} \cdot d^{-1}$ of MHA), and placed on pastures of similar quality. All supplements contained 13.3% protein. Supplementation period for the experiments lasted 54 days in length on average. Across all three experiments no significant difference in weight

changes were observed among cows. In Exp. 3, improved reproductive performance of cows fed 8 and 10 $g \cdot cow^{-1} \cdot d^{-1}$ MHA was observed. Both cow groups fed 8 and 10 $g \cdot cow^{-1} \cdot d^{-1}$ had a 12% or greater increase in number of cows confirmed cycling within the first 21 days of breeding season. Cows from the 8 and 10 $g \cdot cow^{-1} \cdot d^{-1}$ treatment groups also had a 12% or greater increase in conception by artificial insemination compared to the other supplement treatments. The authors noted that cows in Exp. 3 were more nutritionally stressed prior to and following calving compared to the first two experiments due to a more dramatic weight change (Clanton and England, 1980). Clanton and England's (1980) results are similar to that of Nolan et al. (1988) experiment investigating CP requirements, cows fed to meet requirements whether that be AA or CP requirements returned to estrus sooner than those that were still deficient in crude protein or AA.

Varner (1974) published contrasting results to the Clanton and England (1980) study finding no difference in postpartum interval or conception rate of beef cows offered one of three supplemental MHA inclusions at 0, 10, or 15 $g \cdot cow^{-1} \cdot d^{-1}$ for approximately the same supplementation time period while consuming free choice grass hay. Supplement composition in the Varner (1974) experiment contained 14.96% crude protein on a dry matter basis while the MHA supplement in the Clanton and England (1980) study contained 13.3% crude protein. Clanton and England (1980) also noted that cows were more nutritionally stressed before and after calving in Exp. 3, highlighting a larger change in recorded body weights. Given the observations and discussion between the two studies, the differences could be explained through conclusions made by Chandler et al. (1976) in lactating dairy cows; it was determined more of a response could be observed from supplementing MHA if the protein content of the diet was low. Lactating dairy cows fed a diet deficient in crude protein relative to dairy lactation requirements,

including 12.5% crude protein required 2.9 services per conception; whereas cow treatment groups receiving the same 12.5% protein diet supplemented 0.125% MHA and cow treatment groups receiving 15.5% protein diets, both required only 1.8 to 2.2 services per conception, respectively.

In summary, MHA seemed to have a general positive effect on reproductive performance when supplemented to grazing cattle. The differences in results among the conflicting studies may be attributed to the differences in amount of CP available in the diet supplement in addition to, or in combination with the forage provided. The conclusions made by Chandler et al. (1976) noting an increased response may be observed if diets are low in CP further back up this idea. If forage or ration is deficient in CP, addition of MHA to cows through calving season would offer potential for increased reproductive performance during breeding season.

Lactation

During lactation, cows are partitioning much more energy from glucose and nutrients towards milk production for the nursing calf, the plane of nutrition shifts considerably in the time span prior to calving to peak lactation. In high producing lactating dairy cattle this extreme change can lead to ketosis, especially if cattle have low feed intakes immediately following parturition. In a study evaluating the blood lipoproteins in early lactation of Holstein cattle fed a common winter ration, feeding 30 g MHA in conjunction with 20 g ^{L}-methionine intravenous to cattle experiencing ketosis saw rapid improvements in fatty acid composition of total blood serum (McCarthy et al., 1968). They speculated that the rapid return to normal fatty acid composition was due to methionine's key role in the transport of lipids throughout the body. Methionine's key role as a methyl donor in transmethylation reactions during lipid synthesis

could also explain why multiple studies saw an increase in milk fat percentages when milk components were analyzed (Mayes, 1981). Studies by Askonas et al. (1954, 1955) demonstrate that in lactating ruminants milk protein is synthesized from plasma AA and not plasma protein. Thus, ensuring the lactating cow is receiving or is supplemented with the required AA in the diet will aid in optimizing milk production after calving.

Multiple studies have investigated the differences in beef cow milk production and composition at different time points during lactation when cows were supplemented MHA. Varner (1975) evaluated the composition of milk from cattle supplemented a pelleted form of MHA at one of three inclusion levels (0, 5, or 15 $g \cdot cow^{-1} \cdot d^{-1}$) while grazing native range. At roughly 56 days post calving, the butterfat content of milk differed significantly among cows supplemented MHA at 15 $g \cdot cow^{-1} \cdot d^{-1}$ compared to cows receiving no MHA (4.6% butterfat versus 3.8% butterfat; with MHA at 5 $g \cdot cow^{-1} \cdot d^{-1}$ being intermediate. Varner (1975) reported no significant differences in other milk analysis components including percent protein, percent total solids, or percent other solids. The same experiment observed a significant increase in 12-hour milk production between treatments, lactating cows receiving no MHA milked 3.9 kg which was significantly different from cows receiving 10 $g \cdot cow^{-1} \cdot d^{-1}$ that milked on average 4.9 kg. Cows receiving 5 $g \cdot cow^{-1} \cdot d^{-1}$ milked 4.3 kg and were intermediate to the other two treatments. The calculated 4% fat corrected milk from the milk production results followed the same significance pattern described. Similar results were reported in a study by Hess et al. (1998), supplementing a combination of an encapsulated source of lysine and methionine (10 $g \cdot cow^{-1} \cdot d^{-1}$ and 5 $g \cdot cow^{-1} \cdot d^{-1}$) to 2-yr-old cows resulted in increases in milk fat percentage and milk production. Hess et al. (1998) also reported increased milk protein percentages; however, this may be explained by the

experimental treatment diets including CP at 125% of requirements compared to the control treatment.

Varner (1975) contributes the increased milk production to a more efficient use of dietary nitrogen by the cows supplemented with MHA. Prior to the milk production assessment, cows were bled to determine plasma AA levels. Plasma urea and ammonia were significantly lower for treatment groups receiving MHA at 5 g (376.68 $\mu M \cdot L^{-1}$, 221.38 $\mu M \cdot L^{-1}$) and 10 g (322.60 $\mu M \cdot L^{-1}$, 210.05 $\mu M \cdot L^{-1}$) doses compared to cows not receiving MHA (562.14 $\mu M \cdot L^{-1}$, 259.38 $\mu M \cdot L^{-1}$), respectively. Total AA, total essential AA, and total nonessential AA were significantly less for cows fed MHA compared to cows receiving no MHA (Varner, 1975). Less AA concentrations circulating in the blood is supportive of an increase in protein synthesis at the cellular level. These results further support the milk component results indicating that the cattle receiving MHA at 15 $g \cdot cow^{-1} \cdot d^{-1}$ were producing higher quality milk for their nursing calf and would require greater amounts of plasma AA leaving less available AA to circulate in the blood.

Thomas and Langford (1978) conducted a similar experiment to the experiment conducted by Varner (1975), supplementing late gestating cows MHA prior to and after calving. Treatments varied slightly, 6-yr-old Herford × Angus crossbred cows were divided into three treatment groups. Treatments consisted of no supplemental MHA pre- or post-calving, 10 $g \cdot cow^{-1} \cdot d^{-1}$ only after calving, or 10 $g \cdot cow^{-1} \cdot d^{-1}$ supplemented both pre- and post-calving. Like Varner (1974), Thomas and Langford (1978) observed a numerical difference in improved percent butterfat across treatments that received MHA at a rate of 10 $g \cdot cow^{-1} \cdot d^{-1}$ compared to cows that received no MHA supplement, but treatments were not significantly different. Though the differences in butterfat were not statistically significant, the adjusted 205 d calf weaning BW of the calves nursing cows supplemented the 10 $g \cdot cow^{-1} \cdot d^{-1}$ of MHA 30 days pre-calving to

approximately 60 days post-calving, compared to the calves nursing cows supplemented no MHA were significantly heavier (Thomas and Langford, 1978). The increased weaning weights could be partially explained by the increase in available energy and other nutrients provided to the nursing calves from the higher concentrations of butterfat in the milk.

Multiple studies have shown supplementation of MHA to lactating beef cows has offered improvements in milk composition components (Krehbiel et al., 2001; Varner, 1974; Hess et al., 1998); however, only the Varner (1974) and Hess et al. (1998) studies resulted in improvements of overall milk yield. The dairy industry has seen similar significant improvements in milk composition components, especially in milk fat percentage while supplementing MHA to lactating cows (Huber et al., 1984; Holter et al., 1971; Zanton et al., 2014). The same studies noted a numeric difference in milk yield across treatments; however, they were again not significant differences. Only in a study conducted by Griel et al. (1968) supplementing MHA at 40 and 80 g·cow^{-1}·d^{-1} to determine its effects on combating bovine ketosis in lactating dairy cattle, did authors see a significant effect on average weekly milk production between the control treatment supplemented no MHA (193.4 ± 3.3 kg) compared to 40 g MHA·cow^{-1}·d^{-1} (207.5 ± 3.36 kg) and 80 g MHA·cow^{-1}·d^{-1} (196.6 ±3.47 kg). The decrease in milk production between the two treatments receiving MHA was addressed in the article, the authors believed that the reduction was associated with a palatability issue that resulted in lower feed intakes of the 80 g MHA·cow^{-1}·d^{-1} treatment group. The decrease in intakes of cattle supplemented 80 g MHA·cow^{-1}·d^{-1} supports the previously discussed study by Satter et al., (1974) who determined that increasing methionine supplementation above 2.5% of DM can decrease DMI of cattle.

Calf Performance

Gestation and Fetal Nutrition

During late gestation in cattle, maternal anabolism is not likely to occur, meaning that much of the consumed nutrients are partitioned to the developing fetus. Similarly, after birth the source of nutrition provided to the calf to promote growth is supplied solely by the dam in the form of milk. The dam's nutritional needs in late gestation are comprised of nutrients to meet her maintenance requirements in addition to the demands of the fetus. During this time prior to birth, the fetus has a metabolic rate twice that of the dam on a weight-specific oxygen consumption basis (Reynolds et al., 1986). Metabolizable AA requirements for a bovine fetus are three times that of what is required for fetal growth, this points to a significant amount of catabolism of AA that could be used as a fuel source even during short periods of maternal fasting (Bell, 1995; Battaglia, 1992). The partitioning of nutrients towards the fetus is important to ensure proper growth of the fetus during late pregnancy; however, to ensure proper energy and AA supply the partitioning may come at the cost of the lipid and protein stores of the dam (Bell, 1995). Providing supplemental AA to ensure requirements are met for the dam during late gestation is ultimately satisfying the increased fetal demands prior to parturition.

Weaning Weight

Literature strongly supports milk production of the dam is influential on the weaning weight of the calf. Increased preweaning milk intake by the calf tends to result in heavier calf weaning weights and in turn fewer days to reach heavier finals market weights for processing (Abdelsamei et al, 2005). Genetic potential is certainly a factor when considering total milk production; however, proper nutrition to the dam is important to ensure that potential yield can be achieved (Notter et al., 1978; Reynolds et al., 1978; Boggs et al., 1980). Providing the essential AA that are first limiting for milk synthesis allows for the potential to increase production, protein content, and milkfat (Munneke et al., 1991). Increasing the total amount and

nutritional content of the output for the calf would allow more nutrients to be available for growth after maintenance requirements are met.

Previously discussed in the Thomas and Langford (1978) study feeding MHA to cattle pre- and/or post-calving at an inclusion of 10 g MHA·cow^{-1}·d^{-1}, there were numerical differences in milk component analysis, those differences translated into significant differences in calf performance. When 205 d adjusted calf weaning BW were compared across the three treatments (No MHA, 10 g MHA·cow^{-1}·d^{-1} post-calving, or 10 g MHA·cow^{-1}·d^{-1} pre- and post-calving) steers from the treatment cows receiving 10 g MHA·cow^{-1}·d^{-1} both pre- and post-calving were 24.9 kg heavier than the control steers and 16.3 kg heavier than the treatment steers from cows receiving 10 g MHA·cow^{-1}·d^{-1} post-calving at weaning. Feeding MHA to cows improved steer weights more than heifer weights when progeny weaning weights were broken apart by calf sex (Thomas and Langford, 1978).

Similar to Thomas and Langford (1978), in the previously discussed study by Varner (1974), significant differences were observed in weaning weights across treatments. Varner (1974) indicated there were no differences in calf birth weights. However adjusted 205 d BW, actual weaning weight and adjusted ADG were significantly different between calves of cows fed 15 g MHA·cow^{-1}·d^{-1} 30 d prior to calving through 60 d post-calving when compared to calves of cows fed no MHA during that time span. Calves of cows fed 5 g MHA·cow^{-1}·d^{-1} were intermediate but not different between the other two tested treatments. Cows were managed as a common group after supplementation on pasture and calves were weaned at 190 d of age on average. Varner (1974) concluded feeding 15 g MHA·cow^{-1}·d^{-1} 30 d prior to calving and 60 d post-calving increased calf performance measures of ADG and weaning weights by improving milk components provided by the dam.

Contrasting calf performance results were discussed in two additional experiments feeding methionine hydroxy analog to beef cows. Clanton and England's (1980) study consisting of three experiments, Exp. 1 tested a corn-based supplement (13.3% crude protein) and soybean-based supplement (20% crude protein) with varying levels of MHA (0, 5, and 10 g MHA·cow^{-1}·d^{-1}; 0 and 10 g MHA·cow^{-1}·d^{-1} respectively). Experiment 2 and Exp. 3 focused on supplementing MHA at different inclusions in a corn-based supplement (0, 2, 4, 6, 8, or 10 g MHA·cow^{-1}·d^{-1}) for 54 d on average. No milk component analysis was conducted on cows in these experiments making it difficult to determine if milk nutrient content was, in fact, different among treatments. All calf performance measures in Exp. 1 were not significantly different based on the level of MHA inclusion, only differences discussed were the differences between the corn- or soybean-based supplement. Calves from cows fed the 20% crude protein supplement gained more body weight than calves from cows receiving the 13.3% crude protein supplement. Experiment 2 and 3 stated there was no effect of feeding MHA to cows on gains of calves.

Krehbiel et al. (2001) supplemented MHA in a liquid form in combination with additional urea then compared these treatments to supplements with and without additional fat. On spring-calving herds, grazed on range pastures, two experiments were conducted to evaluate the effects of MHA and, or fat in liquid supplements fed to beef cows on range. Experiment 2 will not be discussed because supplements in Exp. 2 were only evaluating additional fat supplemented with urea. However in Exp. 1, a 2 x 2 +1 factorial arrangement was used and treatments included no supplement (NC), liquid molasses-urea based supplement (U), U plus 1.7% MHA (UM), U plus 12% tallow (UF), and U plus 1.7% MHA and 12% tallow (UMF). Supplements were fed to cows confirmed pregnant at 0.91 kg·cow^{-1}·d^{-1} in automated feeders and the supplementation period lasted through calving for 114 d, at the end of supplementation cow-

calf pairs were managed as a common group and calves were weaned on d 231 of the experiment. Contrasts were used to determine the effects of supplements vs. no supplements, supplements with or without methionine, supplements with or without fat, and the interaction between fat and methionine (Krehbiel et al., 2001).

Calves were weighed at d 67 of Exp. 1; there was an interaction between MHA and fat supplementation (Krehbiel et al., 2001). Authors reported that the interaction led to calves from cows supplemented UF weighing 5.8 to 7.7 kg more than calves in other supplement groups, however by the end of the experiment at weaning the only significant difference in calf weaning weights was the comparison between cows supplemented versus cows receiving no supplements. Conclusions from these results indicated that supplementing MHA early may aid in early lactation and offer calf performance advantages; however the advantages of long-term supplementation may be negligible.

Published literature is conflicting in the advantages of supplementing MHA to improve calf performance and weaning weights. Studies that reported improvements in milk composition tended to also report improvements in calf body weights during the experimental period. Initiation, duration, and inclusion level of MHA in supplement differed slightly among experiments making it difficult to isolate an ideal time frame to start and stop supplementation. Forage quality also varied between experiments, difference could be a contributing factor to the level of success or significance that was drawn from the experiments. Indeed if forage quality is adequate in providing CP or essential AA and energy, then supplementation may be deemed unnecessary as a result, in other words the true benefit of supplementing may have been masked. Determining when to initiate supplementation may be contingent on when forage availability or quality decreases, not necessarily a set time period prior to or following calving.

Summary

Cow-calf producers define profitability based on the weight of calves at weaning and their ability to generate the next calf crop in a timely manner. Making sure there are additional pounds generated at weaning requires proper nutrition and management of the cow. Providing essential nutrients, specifically essential AA, that can be most limiting during periods of lactation help to promote improved reproductive performance and increase milk production of the cow. Achieving reproductive improvements can shorten periods of anestrous, allowing for greater instances of subsequent conception. Improvements in cow milk production can be observed in the nursing calf. Improved milk production and composition offer the calf the potential for improved growth leading to increased weaning weights, this may result in greater returns for the cow-calf producer.

Multiple studies in both dairy and beef cattle have determined methionine is the most limiting AA for lactating cows. In light of this discovery, extensive work has been conducted in the dairy industry to determine benefits of supplementing methionine hydroxy analog to improve milk production. Methionine hydroxy analog is a preferred method of supplementing to meet methionine needs due to the analog form's ability to better bypass microbial degradation. Methionine hydroxy analog is a cost effective alternative to supplementing excess methionine or lipid encapsulated forms. Cost effective methods to achieve improved milk production is sought after to increase profits and drive down costs in the dairy industry. Beef production systems in the cow-calf sector, are also concerned with milk production since milk is the sole source of nutrition for the nursing calf. These interests have resulted in beef nutrition research conducted to determine benefits of methionine hydroxy analog supplements offered to cattle that graze low protein forages predominantly in range management systems. There have been observed notable

benefits either in cow reproduction or in increased calf gains. There is still a gap in knowledge of the achievable benefits for cow-calf producers in other regions of the country with different forage based systems. Fall-calving cows grazing predominantly cool-season forages may succumb to amino acid deficiencies due to reductions in forage stands or nutrient content. Identifying the benefits of methionine hydroxy analog in these specific grazing systems may offer additional insights into the application of methionine hydroxy analog and further promote its use in certain U.S. cow-calf systems.

Literature Cited

Abdelsamei, A. H., D. G. Fox, L. O. Tedeschi, M. L. Thonney, D. J. Ketchen, and J. R. Stouffer. 2005. The effect of milk intake on forage intake and growth of nursing calves. J. Anim. Sci. 83: 940-947.

Akobe, K. Z. 1936. Z. Physiol. Chem. 244, 14.

Askonas, B. A., P. N. Campbell, and T. S. Work. 1954. Biosynthesis of Proteins. 2. Synthesis of milk protein by the goat. Biochem. J. 58: 326.

Askonsa, B. A., P. N. Campbell, and T. S. Work. 1955. Biosynthesis of Proteins. 3. Precursors in the synthesis of casein and ß-lactoglobulin. Biochem. J. 61 (1): 105-115.

Bach, A., G. B. Huntington, and M. D. Stern. 2000. Response of nitrogen metabolism preparturient dairy cows to methionine supplementation. J. Anim. Sci. 78: 742-749.

Ballio, A., E. Brandl, E. B. Chain, F. Dentice di Accadi, F. Navagio, and C. Rossi. 1961. The distribution of sulfurylcholine in fungi. Rend. 1st Super. Sanita. 24:366.

Battaglia, F. C. 1992. New Concepts in Fetal and Placental Amino Acid Metabolism. J. Anim. Sci. 70: 3258-3263.

Beal, W. E., D. R. Notter, and R. M. Akers. 1990. Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight gain and postpartum reproduction. J. Anim. Sci. 68(4): 937-943.

Belasco I. 1972. Stability of methionine hydroxy analog in rumen fluid and its conversion in vitro to methionine by calf liver and kidney. J. Dairy Sci. 55(3):353-7.

Belasco, I. J., H. L. Pease, R. W. Reiser. 1978. Microbial conversion of methionine to methionine hydroxy analog and its natural occurrence in various foods and feed products. J Agric. Food Chem. 26(2):327-30.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73: 2804-2819.

Benevenga, N. J. and R. M. Wohlhueter. 1984. Adverse effects of excessive consumption of amino acids. Ann. Rev. Nutr. 4:157-81.

Boggs, D. L., E. F. Smith, R. R. Schalles, B. E. Brent, L. R. Corah, and R. J. Pruitt. 1980. Effects of milk and forage intake on calf performance. J. Anim. Sci. 51: 550.

Campbell B. T., W. M. Backus, C. M. Dixon, R. J. Carlisle, J. C. Waller. 2013. A comparison of spring- and fall-calving beef herds grazing tall fescue. The Prof. Anim. Sci. 29(2):172-8.

Chalupa, W. 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58: 1198-1218.

Clanton, D. C. and M. E. England. 1980. Methionine hydroxy analog in supplements for lactating beef cows. J.Anim. Sci. 51(3):539-43.

Cooper, A. J. 1983. Biochemistry of sulfur-containing amino acids. Annual Review of Biochemistry 52 (1): 187-222.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88 (E Suppl.): E51-E60. doi: 10.2527/jas.2009-2311.

Emanuele, S. M. and D. Putnam. Encapsulating nutrients to improve reproduction and nitrogen utilization in ruminants. Ruminant Nutrition Symposium; February 1-2, 2006; Gainesville FL, USA; 2006. pp. 1-2.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implication for beef cattle production. J. Anim. Sci. 88 (E. Suppl.): E205-E215. doi: 10.2527/jas.2009-2351

Griel, L. C., R. A. Patton, R. D. McCarthy and P. T. Chandler. 1968. Milk production response to feeding methionine hydroxy analog to lactating dairy cows. J. Dairy Sci. 51: 1866.

Harty, A. and K. C. Olson. 2012. Reducing costs of delivering feed to cattle-supplementation frequency. SDSU iGrow Livestock. http://igrow.org/up/resources/02-2048-2012.pdf. Accessed July 27, 2015.

Hess, B. W., E. J. Scholljegerdes, S. A. Coleman, and J. E. Williams. 1998. Supplemental protein plus ruminally protected methionine and lysine for primiparous beef cattle consuming annual rye hay. J. Anim. Sci. 76: 1767-1777.

Hess, B. W., S. L. Lake, E. J. Scholljegerdes, T. R. Weston, V. Nayigihugu, J. D. C. Molle et al. 2005. Nutritional controls of beef cow reproduction. J. Anim. Sci. 83(13 suppl): E90-E106.

Huber, J. T., R. S. Emery, W. G. Bergen, J. S. Liesman, L. Kung, Jr., and K. J. King. 1984. Influences of methionine hydroxy analog on milk and milk fat production, blood serum lipids, and plasma amino acids. J. Dairy Sci. 67:2525-2531.

Holter. J. B., C. W. Kim, and N. F. Colovos. 1971. Methionine hydroxy analog for lactating dairy cows. J. Dairy Sci. 55 (4): 460-465.

Krehbiel, C. R., G. D. Pulsipher, M. K. Petersen, J. E. Sawyer, and E. E. Parker. 2001. Use of urea, ruminally protected methionine, and fat alone or in combination in liquid supplements to enhance nutrient status and milk production in beef cows grazing native range. Novus International Final Report pp 1-24.

Kung, L. and L. M. Rode. 1996. Amino acid metabolism in ruminants. Anim. Feed Sci. Tech. 59: 167-172.

Langer, B. W. 1965. The biochemical conversion of 2-hydroxy-4-methylthiobutyric acid into methionine by the rat in vitro. Biochem. J. 95: 683.

Mayes, P. A. 1981. Metabolism of lipids: II. Role of the tissue. In: D. W. Martin, P. A. Mayes, and V. W. Rodwell (Ed.) Harper's Review of Biochemistry (18th Ed.). pp 222-244. Lange Med. Publ., Low Altos, CA.

Maw, G. A. and C. M. Coyne. 1966. α-Hydroxy acids as metabolites of sulfur amino acids in yeast. Arch. Biochem. Biophs. 177: 499-504.

McCarthy, R. G. Porter, and L. Griel. 1968. Bovine ketosis and depressed fat test in milk: A problem of methionine metabolism and serum lipoprotein aberration. J. Dairy Sci. 51(3):459-62.

Miller, A. J., D. B. Faulkner, R. K. Knipe, D. R. Strohbehn, D. F. Parrett, and L. L Berger. 2001. Critical control points for profitability in the cow-calf enterprise. Prof. Anim. Sci. 17:295-302.

Munneke, R. L., D. J. Schingoethe, and D. P. Casper. 1991. Lactational evaluation of ruminally protected methionine in diets containing extruded soybeans and urea. J. Dairy Sci. 74:227-233.

Nolan, C. J, R. C. Bull, R. G. Sasser, C. A. Ruder, P. M. Panlasigui, H. M. Schoeneman, J. J. Reeves. 1988. Postpartum reproduction in protein restricted beef cows: Effect on the hypothalamic-pitultary-ovarian axis. J.Anim. Sci. 66:3208-17.

Notter, D. R., L. V. Cundiff, G. M. Smith, D. B. Laster, and K. E. Gregory. 1978. Characterization of biological types of cattle. VII. Milk production in young cows and transmitted and maternal effects on preweaning growth of progeny. J. Anim. Sci. 46: 908.

Rode, L. 2014. Methionine and more, production response and economics. In: Penn State Dairy Cattle Nutrition Workshop. Pennsylvania State University, State College, PA. p. 1-6.

NRC 1996. Nutrient Requirement of Beef Cattle. 7th ed. National Academy Press, Washington, DC.

Patterson, H. H., D. C. Adams, T. J. Klopfenstein, R. T. Clark, and B. Teichert. 2003. Supplementation to meet metabolizable protein requirements of primiparous beef heifers: II. Pregnancy and economics. J. Anim. Sci. 81: 563-570.

Patterson, J. A. and L. Kung, Jr. 1988. Metabolism of DL-methionine and methionine analogs by ruminal microorganisms. J. Dairy Sci., 71: 3292-3301.

Ramsey, R., D. Doye, C. Ward, J. McGrann, L. Falconer, S. Bevers. 2005. Factors affecting beef cow-herd costs, production, and profits. Journal of Agricultural and Applied Economics 37(01):91-9.

Randel R. D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853-62.

Reynolds, W. L., T. M. DeRouen, and R. A. Bellows. 1978. Relationships of milk yield of dam to early growth rate of straightbred and crossbred calves. J. Anim. Sci. 47: 584.

Richardson, C. R. and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46: 740-745.

Roberts, C. A., G. D. Lacefield, D. Ball, and G. Bates. 2009. Management to optimize grazing performance in the northern hemisphere; agronomy monographs 53. In: Tall Fescue for the 21st Century. Madison, WI: American. Society of Agronomy, Crop Science Society of America, Soil Science Society of America. pp 85-99.

Rutledge, J. J., O. W. Robison, W. T. Ahlschwede, and J. E. Legates. 1971. Milk yield and its influence on 205-day weight of beef calves. J. Anim. Sci. 33(3):563-7.

Satter, L. D., R. L. Lang, J. W. Van Loo, M. E. Carlson, and R. W. Kepler. 1974. Adverse effect of excess methionine or methionine hydroxy analog on feed consumption in cattle. J. Dairy Sci. 58 (4): 521-525.

Sasser, R. G., R. J. Williams, R. C. Bull, C. A. Ruder, and D. G. Falk. 1988. Postpartum reproductive performance in crude protein-restricted beef cows: return to estrus and conception. J. Anim. Sci. 66: 3033-3039.

Schwab, C. G. and R. S. Ordway. 2003. Methionine supplementation options. In: Proc. Four-State Applied Nutrition and Management Conf., July 9-10, La Crosse, WI, p. 93-98

Smith, S. and J. Boling. 1984. Lipid coating as a mode of protecting free methionine from ruminal degradation. J. Anim. Sci. 58(1):187-93.

Thomas, O. O. and W. J. Langford. 1978. Methionine hydroxy analog in Beef Cow Supplements given pre- and post calving. Proc. West. Sec. Amer. Soc. Anim. Sci. 29:454-455.

Varner, L. W. 1974. Supplementary diets of beef cows with methionine hydroxy analog. Montana Agr. Exp. Sta. Res. Rep. 52:34.

Waterman, R. C., C. A. Löest, W. D. Bryant, M. K. Petersen. 2007. Supplemental methionine and urea for gestating beef cows consuming low quality forage diets. J. Anim. Sci. 85(3):731-6. doi: 10.2527/jas.2006-425

Wettemann, R. P., C. A. Lents, N. H. Ciccioli, F. J. White, and I. Rubio. 2003. Nutritional- and suckling-mediated anovulation in beef cows. J. Anim. Sci. 81 (2): E48-E59.

Wu, S. H. W. and A. Papas. 1997. Rumen-stable delivery systems. Adv. Drug Deliv. Rev. 28(3):323-34.

Zanton, G. I., G. R. Bowman, M. Vázquez-Añón, and L. M. Rode. 2014. Meta-analysis of lactation performance in dairy cows receiving supplemental dietary methionine sources or postruminal infusion of methionine. J. Dairy Sci. 97: 7085-7101. doi: 10.3168/jds.2014-8220

CHAPTER 2

EFFECTS OF SUPPLEMENTING METHIONINE HYDROXY ANALOG ON COW AND CALF PERFORMANCE

Abstract

Mature Simmental × Angus cows (214 cows; 635 ± 7.4 kg) were utilized to determine the effects of late gestation and early postpartum supplementation of methionine hydroxy analog (MFP; Novus International, Inc., St. Charles, MO, USA) on cow BW, BCS, milk production, milk composition, reproduction, and calf performance until weaning in a fall-calving, cool-season grazing system. Cows were confirmed pregnant prior to experiment, stratified by BW, cow age and AI sire, and assigned to one of twelve pastures (17 or 18 cows·pasture^{-1}). Pastures were randomly allotted to one of two treatments: control (0.45 kg·cow^{-1}·d^{-1} of wheat mid-based pellets, n = 6) or supplement including MFP (0.45 kg·cow^{-1}·d^{-1} of wheat mid based pellets including 10 g MFP, n = 6). Treatments were fed 23 ± 1 d prior to calving through 72 ± 1 d postpartum. Cows were weighed post-calving, at supplementation end, AI breeding, AI pregnancy check, and the end of trial (192 and 193 ± 1 d postpartum). At 74 d postpartum, a subset of cow-calf pairs were used in a weigh-suckle-weigh to determine milk production and milk samples were taken to determine milk composition (n = 45·treatment^{-1}). A serum P_4 assay was utilized to determine cow cyclicity. After supplementation, all cow-calf pairs were managed as a common group until weaning (191 ± 1 d of age). Cows were bred via AI at 97 ± 1 d postpartum and clean-up bulls were turned out 11 d post-AI for a 55 d breeding season. Cow and calf performance were analyzed using MIXED procedure in SAS (SAS Inst. Inc., Cary, NC) and cow reproductive performance and calf health were analyzed using GLIMMIX procedure. Cow BW and BCS were not different ($P \geq 0.10$) at any time points between treatments. There was no treatment effect ($P \geq 0.62$) on calf birth BW, calf weaning BW (191 ± 1 d of age), or calf ADG.

Calculated 24 h milk production did not differ ($P = 0.76$) nor did milk composition or component production ($P \geq 0.21$). There was no difference ($P = 0.69$) in percentage of cows cycling between treatments. Cow AI conception rate and overall pregnancy rate were not different ($P \geq 0.50$) between treatments. No differences ($P \geq 0.61$) in calf health were observed. Supplementation of MFP during late gestation through estimated peak lactation did not affect cow performance, cow milk production, or calf performance when fall-calving cows grazed cool-season forages.

Key Words: cow-calf, fall-calving, late gestation supplementation, methionine hydroxy analog, milk production, reproduction

Introduction

Increasing weaning weights continues to be a goal for producers and, since milk production of the dam has a significant effect on calf growth, is important to enhance production (Rutledge et al., 1971; Beal et al., 1990). During lactation, reproductive concerns may arise and maintaining ideal reproductive performance increases overall lifetime profitability of the dam (Patterson et al., 2003). Managing dam nutrition after calving is key to allow for ideal performance, both for optimal milk production and subsequent reproduction. Undernutrition of protein and energy in late gestation and lactation may result in longer anestrus and decreased fertility (Meteer et al., 2015). Grazing cattle obtain the majority of these requirements from rumen microbial protein; however, further supplementation may be needed (Klopfenstein, 1996). The first limiting amino acid in grazing and lactating dairy and beef cows is methionine (Waterman et al., 2007; Richardson and Hatfield, 1978; NRC, 2001) and evidence suggests there are increased methionine requirements for gestating cattle (Bach et al., 2000).

Methionine supplement comes in many forms; however, free amino acid sources are costly and unjustified when other rumen-protected forms are available (Kung and Rode, 1996). Protected methionine forms with physical coatings of lipid and pH sensitive polymers or analog forms bonded to hydroxyl or isopropanol groups have been effective in reducing the need for increased CP in the diet while improving N utilization in dairy cows (Chen et al., 2011). Methionine hydroxy analog (MHA) is a source of rumen-protected methionine that positively affects microbial protein synthesis and metabolism (Lee et al., 2015). Clanton and England (1980) reported increased conception rates in cows supplemented MHA. Varner (1975) observed increased milk production in lactating cows fed MHA on range. No research has explored supplementing MHA to fall-calving cows grazing cool-season forages. The objectives of the present study were to evaluate the effects of MHA supplementation pre- and postpartum on cow performance, milk production, reproduction, and calf performance. The hypotheses were that cows supplemented MHA would yield increased milk outputs and, therefore, increased calf gains. In addition, it was hypothesized that these fall-calving cows receiving MHA would have improved reproductive performance while grazing cool-season forages.

Materials and Methods

Cattle used in this trial were managed according to the guidelines recommended in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Consortium, 1988). All experimental procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee.

Animals and Experimental Design

Two hundred fourteen late-gestation Angus × Simmental cows (mean ± SD; BW 635 ± 7.4 kg) from Dixon Springs Agricultural Research Station in Simpson, IL were utilized to evaluate the effects of supplementing methionine hydroxy analog (**MHA**) prior to and following

parturition on cow performance, milk production, milk composition, conception rate, and calf performance.

Cows were stratified by BW, cow age, and AI sire and assigned to one of twelve pasture groups (17 or 18 cows·pasture^{-1}) and randomly allotted to one of two supplemental treatments: control (**CON**; 0.45 kg·cow^{-1}·d^{-1} of wheat mid-based pellets) or 0.45 kg·cow^{-1}·d^{-1} of wheat mid-based pellets including 10 g of a methionine hydroxy analog (**MFP**; (Novus International, Inc. St. Charles, MO, USA). Cows were confirmed pregnant by ultrasound (Aloka 500 instrument (Wallingford, CT); 7.5 MHz general purpose transducer array; February 10th and 12th, 2014) and palpation methods (August 19th, 2014), to AI with one of sixteen Angus or Charolais sires. Supplementation began on August 20th, 2014, 23 d ± 1 d prior to average calving date (September 12th, 2014). All cows grazed cool-season pastures which predominately included endophyte-infected tall fescue (*Festuca arundinacea* cv. Kentucky 31), red clover (*Trifolium pretense*), and white clover (*Trifolium repens*) and had ab libitum access to a salt and mineral block (salt = 21.4%, Ca = 16.9%, Na = 8.5%, P = 7.1%, Mg = 5.9%, K = 1.1%, Cu = 997 mg·kg^{-1}, Se = 25.9 mg·kg^{-1}, vitamin A = 109 kIU·kg^{-1}, chlortetracycline = 5.7 g·kg^{-1}) during the 96 d supplementation period ending November 24th, 2014 (74 ± 1 d postpartum). Average stocking rate during the supplementation period was 0.33 ± 0.14 hectares per cow with pasture size averaging 5.82 ± 2.37 hectares. Pasture groups were rotated under the discretion of trained University of Illinois research personal based off visual appraisal of forage availability. When a pasture group was rotated due to limited forage availability, a pasture group from the alternative treatment was also rotated to a new pasture. Following the conclusion of the supplementation period, on November 25th, 2014 (75 ± 1 d postpartum), cow-calf pairs were commingled and managed as a common group until weaning. Cow-calf pairs grazed stockpiled tall fescue pastures

with ad libitum access to a salt and mineral block. During the commingled period, all cows were supplemented (4.5 $kg \cdot^{-1} \cdot d^{-1}$) of a supplement consisting of 50% shelled corn (ADF 2.8%, NDF 9.6%, CP 7.8%, crude fat 3.6% on a DM basis) and 50% DDGS (ADF 10.5%, NDF 33.1%, CP 26.3%, crude fat 8.2% on a DM basis). Cows also had access to free choice hay (ADF 35.5%, NDF 64.9%, CP 4.5%) when forage stands were depleted. Forage (ADF 24.4%, NDF 50.4%, CP 11.1%) during the commingled period was stockpiled tall fescue. The supplementation during the commingled period began December 24th, 2014 and continued until March 24th, 2015. Pasture size averaged 22.94 ± 9.22 hectares with an average stocking rate of 0.19 ± 0.05 hectares per cow during the commingled period. Calves were weaned March 24th, 2015 (191 ± 1 d of age).

Cow Performance

At the initiation of the experiment, consecutive two-day full BW and BCS were taken on all cows. A one-day mid-point full BW and BCS was taken on all the cows post-calving (27 ± 1 d postpartum). Following the end of the supplementation period and prior to commingling treatment groups, a one-day shrunk BW and BCS was taken on the cows (72 ± 1 d postpartum). One-day full BW and BCS were taken prior to breeding (94 ± 1 d postpartum), at AI breeding (97 ± 1 d postpartum), and at AI pregnancy check (132 ± 1 d postpartum). A final consecutive, two day full BW and BCS were taken on all cows at the end of the trial (192 and 193 ± 1 d postpartum).

Milk production and composition

Following the conclusion of supplementation (November 24th, 2014; 74 ± 1 d postpartum) milk production and composition were evaluated on a subset of cows from each treatment group. A random subset of 8 cow-calf pairs from each treatment pasture were used in

the weigh-suckle-weigh (WSW) procedure and milk composition analysis. Cows were administered 1 mL·cow^{-1} oxytocin (Oxoject, Henry Schein Animal Health, Dublin, OH, USA) to stimulate milk let down and then were hand milked to obtain a 50 mL sample for milk composition analysis. Prior to sample collection, cow teats were cleaned with a clean cloth and 70% isopropyl alcohol, and the teat was hand-stripped approximately three times prior to collecting milk for sample analysis. Individual samples were strained through a clean 2-layer piece of cheesecloth (VWR International, Radnor, PA, USA) to remove any foreign dirt or hair prior to filling the milk sample tube. Milk samples were stored in capped plastic tubes provided by Dairy Lab Services (Dubuque, IA, USA) containing potassium dichromate preservative ($Kr_2Cr_2O_7$) to maintain milk quality until arrival to the lab for infrared instrumentation analysis (Ng-Kwai-Hang and Hayes, 1982). Milk sample collection began at 10.5 h after supplementation and was completed 13.5 h after supplementation. Milk samples were refrigerated overnight (< 4°C) and shipped to Dairy Lab Services the following morning. After milk sample collection was complete, cow-calf pairs were reunited by pasture group to nurse and to allow for the start of the 12 h milk production measure (Beal et al., 1990). Cow-calf pairs were again separated after nursing was complete. Nursing order alternated between treatment groups to reduce variation. Calves and cows were separated 12 h and, the following morning, calves were weighed to record an empty BW. Calves were then reunited with their mothers and, immediately following nursing, the calves were weighed again to record a full BW. The difference in BW from full and empty calf BW are representative of 12 h milk production of the cow. The 12 h milk production was doubled to estimate 24 h milk production.

Reproductive Performance

Blood was collected from all cows to determine serum progesterone concentrations. The first blood collection occurred at the end of the supplementation period and the second collection occurred ten days later (72 ± 1 d postpartum and 82 ± 1 d postpartum, respectively). Blood was collected by means of a 12 mL syringe (Monoject, Covidien, Mansfield, MA) via the jugular vein, and stored in a 10 mL borosilicate glass tube (Thermo Fisher Scientific Inc., Waltham, MA) where it was allowed to clot at room temperature prior to being centrifuged at 1,300 × *g* for 20 min at 5°C (Thermo Fisher Scientific Sorvall Centrifuge, Waltham, MA, USA). Serum was then stored at -20°C for later analysis. Serum progesterone concentrations were analyzed by solid-phase, competitive, chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Estrous cyclicity was then determined when serum progesterone concentrations met or exceeded 1 $ng \cdot mL^{-1}$ on either collection time point.

Cows were synchronized using the Co-Synch+CIDR procedure prior to breeding (87± 1 d postpartum) (Bremer et al., 2004), and artificially inseminated to one of six AI bulls (97 ± 1 d postpartum). Ten d following AI, cows were exposed to six bulls for a 55 d breeding season. Conception to AI and overall pregnancy were determined at 34 d post-AI service and 96 d post-AI service, respectively. AI conception and overall pregnancy rates were determined by a trained technician via ultrasonography (Aloka 500 instrument (Wallingford, CT); 7.5 MHz general purpose transducer array). Cow embryonic loss was determined at the overall pregnancy check and cow fetal loss was determined the following fall calving season. Cows that were confirmed AI pregnant at the first pregnancy check and were open or not determined 95 d pregnant at the overall pregnancy check were recorded as an embryonic loss. Cows that did not calve the following fall after being confirmed pregnant by AI at both previous palpation dates or were confirmed pregnant at the overall pregnancy check were recorded as an observed fetal loss.

Calf Management and Data Collection

Calf BW was collected using a hand scale within 48 h of birth. A subset of calves (8 calves per pasture group) were weighed at the end of the supplementation period during the WSW procedure (72 ± 1 d of age; 96 total calves, 51 steers, 45 heifers). All calves were weighed for a preweaning mid-point one-day full BW (93 ± 1 d of age). At weaning, a consecutive two-day full BW was taken on all calves (191 ± 1 d and 192 ± 1 d of age). Prior to weaning, all calves were vaccinated with Bovi-Shield Gold FP 5 VL5 (Zoetis, Florham Park, NJ, USA), Covexin 8 (Merck Animal Health, Madison, NJ, USA), and MpB Guard Mycoplasma Bovis Bacterin (AgriLabs, St. Joseph, MO, USA). Calf performance was reported for all calves (174 total calves, 82 steers, 91 heifers) born to cows placed on one of the two dietary treatments that were weaned on d 214 of the trial, minus calves that were injured or deceased prior to d 214. Calf health was monitored by trained university farm personal. Calves were removed from the study for mortality prior to weaning (15 calves) or dam removal due to death, lameness, or chronic illness during the experiment (19 calves). Cows were also removed from trial that did not have a recorded calf during the experiment (6 cows).

Feed Sampling and Analysis

Forage samples were collected once monthly from the pastures where cows grazed during the supplementation period and commingled period until weaning. Additional forage samples were collected on day of pasture movement. One sample was taken from the pasture the cows were previously grazing and one sample was taken from the new pasture the cows were moved to.

Treatment supplements were sampled four times over the supplementation period, at the initiation of supplementation (trial d 0), at the end of first supplement load (trial d 62), delivery

of second supplement load (trial d 63), and at the end of supplementation (trial d 94). Supplemented feed provided to the cows once they were commingled were also sampled every 28 d. Forage samples were composited by pasture group during the supplementation period. Forage and hay were also composited during the commingled period. All feed and forage samples were dried at 55^0C for 3 d and then ground using a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). All samples were composited appropriately by treatment pasture and/or experimental period. All samples were then analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), total ash (600°C for 2 h, Thermolyne muffle oven model: F30420C, Thermo Scientific, Waltham, MA), and ADF and NDF (using Ankom Technology method 5 and 6, respectively; Ankom^{200} Fiber Analyzer, Ankom Technology, Macedon, NY). Cow treatment samples and supplement ingredients fed during the commingled period were also analyzed for crude fat (ether extract method; Ankom Technology).

Statistical analysis

The MIXED procedure (SAS Version 9.4, SAS Inst. Inc., Cary, NC) was utilized to analyze all variables excluding cow reproductive performance, calf morbidity, and calf mortality. Pen was used as the experimental unit. Fixed effects included treatment and cow age in the model statements for all variables for cows and calves, excluding calf morbidity and mortality. The AI sire was included as a fixed effect in the models for all variables pertaining to calf performance. Least square means function of SAS was used to separate treatment means. The GLIMMIX procedure (SAS Version 9.4, SAS Inst. Inc., Cary, NC) was utilized to analyze cow reproductive performance (cyclicity, AI conception rate, overall pregnancy rate, and embryonic loss) and calf morbidity and mortality using binomial distributions. Significance was declared at $P \leq 0.05$ and trends will be discussed at $0.05 < P \leq 0.10$.

Results and Discussion

Forage and Supplement Analysis

Nutrient composition of pasture forages (Tables 1 and 2) during the supplementation period were not different among any tested parameters between treatments ($P \geq 0.36$). According to the NRC (1996), cattle used in this study (assuming the requirement of cows weighing 635 kg with peak milk of 4.5 kg) were provided adequate CP on a kg·d^{-1} basis, from grazing treatment pastures during the supplementation period (1.11 kg DM·d^{-1}). However, CP requirements in kg·d^{-1} are based on estimates of DM intakes. Intakes were not recorded in this study which makes it difficult to determine if requirements were truly met on forage alone. The NRC assumes cow DMI is approximately 2% of BW; however, if cow intake was less than the assumed intake, the CP requirements in kg·d^{-1}may not be met. If cows only consumed 1.5% or 1.75% of BW, the CP intake based on the forage analysis would only be 0.87 kg·d^{-1} or 1.01 kg·d^{-} pre-calving, respectively. Under conditions where actual cow DMI was less than that of NRC estimates, CP would have been limiting among cows grazing treatment pastures during the experiment. After supplementation ended, cows' CP requirements (0.95 kg·d^{-1} for 544 kg cows with 4.5 kg peak milk, 3 months post-calving) were also assumed to be met grazing the stockpiled common pasture (NRC, 1996). The NRC assumes cows at 3 months post-calving have DMI of 2.16% of BW. However, if cows have intakes less than the assumed intake, CP intake requirements would again be greater. If cow intakes were 1.5% or 1.75% of BW, the CP intake would be 0.91 kg·d^{-1} and 1.06 kg·d^{-1}, respectively. Cows grazing common pasture after supplementation ended may not have been meeting CP requirements if cow DMI was only 1.5% of body weight.

Previous studies evaluating effects of supplementing methionine hydroxy analog indicated cattle were grazed on range pasture settings which are typically lower in CP than the

cool-season forages utilized in this experiment (Thomas and Langford, 1978; Varner, 1974). In previous research conducted on range systems, cows also received a variety of hay for varying durations in addition to the standing forage available which differed from the current study (Clanton and England, 1980; Thomas and Langford, 1978; Varner, 1974). Although pasture forage analysis was not provided in those studies, one could speculate that CP protein requirements of the dams were met. The increased CP of the forage may mask any effects of additional AA supplementation since the requirements may have already been met. This idea of masking the AA response due to CP content of the forage was suggested by Krehbiel et al. (2001). The forage quality in Krehbiel et al. (2001) experiment increased in CP content during the experimental period, which is typical of spring forage seasonal growth. Krehbiel et al., (2001) noted the potential for economic advantage to supplementing methionine hydroxy analog early in lactation when the forage quality was lower in early spring. The economic advantage of supplementing MHA continued to decrease as the forage improved up until the end of the 114 d supplementation period (Krehbiel et al., 2001). In this study, however, the CP content was comparable from the supplemental period to the commingled period.

Cow Performance

Cow BW and BCS data are shown in Table 3. As expected by design, there was no difference in BW between treatments at the initiation of supplementation ($P = 0.68$). As BW did not differ at the beginning of the experiment, neither did cow BCS ($P = 0.10$). At 28 d ± 1 d postpartum there was no difference in cow BW ($P = 0.41$) or cow BCS ($P = 0.51$). One-day shrunk BW and BCS of cows taken at the conclusion of the supplementation period did not differ ($P \geq 0.76$). Similarly, cow BW and BCS at breeding did not differ ($P \geq 0.62$). At time of AI pregnancy check, cow BW and BCS were not different ($P \geq 0.67$). At the conclusion of the

experiment when calves were weaned, no differences were observed in cow BW or BCS ($P \geq$ 0.36). No significant differences, due to supplementation of MHA, in cow BW and BCS were observed in other experiments over the duration of supplementation (Clanton and England, 1980; Varner, 1975; Thomas and Langford, 1978; Wright et al., 1975). In contrast, Krehbiel et al. (2001) did note there were cow BW change differences between the supplementation period and the grazing period among treatments; however, at the end of the experiment, there were no differences in overall cow BW change. Krehbiel et al. (2001) attributed the significance of the BW changes to compensatory gain due to increased forage quality earlier in the grazing season in Exp. 2 in comparison to forage quality in Exp. 1 in their discussion. However, Thomas and Langford (1978) and Clanton and England (1980) reported cows numerically gained weight by the conclusion of the research trial which differed from the present trial as cows continued to lose weight until weaning in our experiment. Differences in the time of year or season may have had effect on the differences in BW gains. Cows from previous studies (Clanton and England, 1980; Varner, 1975; Thomas and Langford, 1978; and Krehbiel et al. (2001) were spring calving cows, spring forage quality tends to increase as the season progresses. Cows in the present study calved in the fall, fall forage quality tends to decline as the season progress. Differences in cow BW gains may have been partially attributed to seasonal conditions.

No significant differences in weight changes across previous experiments would potentially indicate that the supplemental methionine was generally not utilized for cow BW gain. The previous research with similar cow performance results would suggest that the AA, such as methionine were being partitioned for milk synthesis. Cow BW loss would indicate supplementation was required; however, the parameters measured did not indicate that supplementation of methionine hydroxy analog offered any increased performance. We speculate

that energy was also limiting due to the continued decrease in cow BW, and if additional energy was also included in addition to the supplemental treatments we may have observed a different response. As stated previously, cows in previous research on range received varying amounts of supplemental hay during the experiment which could have some impact on the differences in performance results.

Milk Production and Composition

Cow milk production and composition analyses are shown in Table 4. Milk production did not differ between treatments ($P = 0.76$). Milk production was approximately 1 kg·d^{-1} lower than previously reported for this herd (Shoup et al., 2015). We speculate that energy intake may have limited milk production as well. There were no differences in any milk component parameters measured and, in turn, no differences in 24 h milk component production ($P \geq 0.21$). Results of this study differ from previous experiments. Varner (1974) reported increased milk production for cows receiving MHA at 5 g·cow^{-1}·d^{-1} and 15 g·cow^{-1}·d^{-1} compared to the control cows receiving no MHA. In addition, Varner (1974) noted a significant difference in 4% fat corrected milk between control cows and cows receiving MHA at 15 g·cow^{-1}·d^{-1}, cows receiving MHA had increased 4% fat corrected milk. Thomas and Langford (1978) and Krehbiel et al., (2001) reported no differences in milk production and milk composition between control treatments receiving no MHA and treatments with MHA included in the supplement. Estimated average milk production was numerically similar to the results of this study. There were no differences ($P \geq 0.68$) in milk component production per day. In dairy cattle, supplementing a rumen protected methionine increased milk production-related traits compared to control diets potentially by meeting essential AA requirements delivered in MP (Osorio et al., 2013). Differences in results across previous research and results from this study could be attributed to

differences in cattle breeds, addition of hay included with the forage source, and the inclusion rate of MHA in the supplement.

Calf Performance

Calf performance data from birth through weaning are shown in Table 5. There was no difference ($P = 0.91$) of calf birth BW between treatments. Varner (1974) reported similar results to the current study; calf birth BW did not differ among treatments when adjusted on a male calf basis. The fetus has an increased requirement for AA; however, previous literature suggests that increased demands do not necessarily correspond to increased protein deposition (Bell, 1995; Battaglia, 1992). In summary, it was not surprising there were no differences in calf birth BW between treatments in this study.

Foreshadowed by the milk production and composition analysis, there were no differences in calf performance measures. The subset of calves weighed during the WSW ($45 \cdot \text{treatment}^{-1}$) did not differ in BW ($P = 0.69$) or midpoint BW ($P = 0.62$) of all calves taken at 94 ± 1 d of age. Calf BW, taken at 191 ± 1 d, were again not different ($P = 0.63$) among treatments. Health status of calves in terms of morbidity and mortality did not differ ($P \geq 0.61$). Calculated calf ADG (Table 6) were not different ($P \geq 0.17$) over the course of experiment. In studies where calf performance differences were observed, there were, at minimum, substantial numeric differences in milk component analysis, particularly milk butterfat percentages. Thus, authors attributed the calf performance differences to improved cow milk composition (Thomas and Langford, 1978; Varner, 1974). Increased butterfat production per day have been positively associated with to increased calf ADG among crossbred beef cows (Belcher and Frahm, 1979; Chenette and Frahm, 1981). Calves receiving more nutritionally dense milk are able to grow at an increased rate compared to those who do not receive the same quality milk when nursing the

dam even if quantity is greater (Brown and Brown, 2002). Similarly, Clanton and England (1980) observed no differences in calf gains or weaning BW among the calves born to dams supplemented MHA. Unlike the results of this study and the Clanton and England (1980) study, Thomas and Langford's (1978) study reported calf weaning BW was improved among treatment groups of cows supplemented with MHA pre- or post-calving. Varner (1974) also reported increased ADG and weaning BW among calves when cows were supplemented MHA at a level of 15 $g \cdot cow^{-1} \cdot d^{-1}$ compared to cows that received the control treatment with no MHA included.

Reproductive Performance

Cow cyclicity at the end of supplementation and other reproduction performance measures are shown in Table 6. Supplement treatments had no effect ($P \geq 0.67$) on cyclicity or AI conception (Table 6). Prior to synchronization and breeding, 74% of control treatment cows were cycling verses 77% of MFP treatment cows ($P = 0.69$). AI conception rate was not different ($P = 0.67$) between treatments. Clanton and England (1980) reported contrasting results in Exp. 3 of their study. With increasing concentrations of MHA included in the supplement, there was a significant increase in percent cycling. Clanton and England (1980) also reported a 12% or greater increase in AI conception rate of cows fed 8 or 10 $g \cdot cow^{-1} \cdot d^{-1}$ in comparison to those receiving lower concentrations of MHA. In the Clanton and England (1980) discussion, authors mention that cows were more nutritionally stressed in Exp. 3 compared to Exp. 1 and Exp. 2 where no significant differences in reproductive performance where observed among MHA treatments. Cows in Exp. 3 continued to lose weight during lactation post-calving across all MHA inclusion treatment levels. In contrast, cows in Exp. 1 and 2 had small numeric gains during the same period post-calving (Clanton and England, 1980). Comparisons with other

studies (Varner, 1974, Thomas and Langford, 1978, and Krehbiel et al., 2001) are not possible because they did not report AI conception rate.

Cow BCS at time of breeding averaged 4.8 ± 0.16 for cows that received CON and 4.5 ± 0.11 for cows that received MFP. Ideally a BCS ≥ 5 has been generally accepted as an adequate threshold for optimum reproductive performance (Meteer et al., 2015). Results of BCS at time of breeding would indicate that cows on both treatments were nutritionally challenged. There were no differences ($P = 0.50$) in overall pregnancy rate at time of weaning between the CON and MFP (78% and 73%, respectively). Varner (1974), Thomas and Langford (1978), and Krehbiel et al., (2001) also reported no differences in overall pregnancy rate between treatments. However, these studies reported overall pregnancy rates numerically greater than results of the present study. Results of the present study would suggest that the opportunity for reproductive improvement did exist since pregnancy rates were lower than previous research and industry averages. Cow embryonic loss and fetal loss were not different ($P \geq 0.66$) between treatments.

Supplementing methionine hydroxy analog pre- and postpartum to fall-calving cows while grazing cool season forages resulted in no effects on cow performance, milk production and composition, or cow reproductive performance. Since milk production and composition was not affected by methionine hydroxy analog supplementation, it was not surprising that calf performance did not differ either.

This data suggests that pre- and post-partum supplementation of methionine hydroxy analog would not provide significant additional performance to fall-calving cows grazing cool season pastures or improve performance of the calves through normal weaning.

Tables

Table 1. Nutrient composition (DM basis) of pastures during supplementation

	Treatments			
Item	CON	MFP	SEM	*P*-value[1]
DM, %	34.5	34.6	2.0	0.93
NDF, %	54.4	55.3	1.2	0.64
ADF, %	28.1	28.5	0.9	0.76
CP, %	8.9	9.5	1.1	0.72

[1]Forage analysis during supplementation period, n = 6 pen·treatment^{-1}

Table 2. Nutrient composition (DM basis) of wheat mid supplement fed during 96 d supplemental period

Item	Treatments[1,2]	
	CON	MFP
DM, %	88.3	88.3
NDF, %	26.4	25.0
ADF, %	7.5	7.3
CP, %	14.1	12.1
Crude fat, %	2.8	3.6

[1] Ad libitum access to block mineral: salt = 21.4%, Ca = 16.9%, Na = 8.5%, P = 7.1%, Mg = 5.9%, K = 1.1%, Cu = 997 $mg \cdot kg^{-1}$, Se = 25.9 $mg \cdot kg^{-1}$, vitamin A = 109 $kIU \cdot kg^{-1}$, and chlortetracycline = 5.72 $g \cdot kg^{-1}$.

[2] Supplement was provided at 0.45 $kg \cdot cow^{-1} \cdot d^{-1}$

Table 3. Effects of supplementation of MFP on cow BW and BCS

Item		Treatments		SEM	*P*-value
		CON	MFP		
n, pen		6	6	-	-
BW, kg					
	Initial[1]	636	634	7.4	0.68
	Post-Calving[2]	645	638	9.5	0.41
	End of Supplementation[3]	563	562	12.6	0.91
	Breeding[4]	566	559	10.7	0.61
	Pregnancy Check[5]	559	555	5.5	0.71
	Weaning[6]	510	500	10.4	0.36
BCS					
	Initial[1]	5.8	5.9	0.1	0.10
	Post-Calving[2]	5.7	5.8	0.1	0.51
	End of Supplementation[3]	5.2	5.2	0.2	0.76
	Breeding[4]	4.5	4.5	0.1	0.82
	Pregnancy Check[5]	4.4	4.4	0.1	0.91
	Weaning[6]	3.9	3.9	0.1	0.65

[1] Trial d 0 two-day average weight
[2] Trial d 51 one-day full weight taken, 28 d ± 1 d after average calving date
[3] Trial d 98 shrunk weight taken
[4] Trial d 121 one-day full weight taken
[5] Trial d 156 one-day full weight taken
[6] Trial d 217 two-day average weight

Table 4. Effects of supplementation of MFP on milk composition and component production

	Treatments			
Item	CON[1]	MFP[1]	SEM	*P*-value
n, pen	6	6	-	-
Milk production[2], kg·d^{-1}	5.94	5.76	0.48	0.76
Fat, %	1.69	1.82	0.17	0.50
Fat, kg·d^{-1}	0.10	0.10	0.01	0.92
Lactose, %	4.85	4.86	0.15	0.94
Lactose, kg·d^{-1}	0.29	0.28	0.03	0.68
Protein, %	3.10	3.27	0.18	0.41
Protein, kg·d^{-1}	0.18	0.18	0.02	0.88
Other solids, %	5.76	5.78	0.14	0.89
Other solids, kg·d^{-1}	0.34	0.33	0.03	0.69
Total solids, %	10.50	10.85	0.20	0.21
Total solids, kg·d^{-1}	0.63	0.62	0.05	0.86
MUN, mg·dL^{-1}	15.85	14.97	1.16	0.58

[1] Subset sample (45·treatment^{-1})

[2] Milk production determined via weigh-suckle-weigh technique at 74 d postpartum on a subset of cows

Table 5. Effects of supplementation of MFP on calf performance and calf ADG

	Treatments			
Item	CON	MHA	SEM	*P*-value
n, pen	6	6	-	-
Calf birth BW, kg	35	35	1.2	0.91
Calf WSW BW[1], kg	104	106	4.5	0.69
Calf 94 d BW, kg	114	116	4.1	0.62
Calf weaning BW[2], kg	148	150	4.9	0.63
Morbidity, %	27.2	27.8	1.1	0.61
Mortality, %	28.5	28.7	1.2	0.89
Calf ADG				
Birth to WSW[1]	0.95	0.98	0.10	0.50
WSW to 94 d BW[1,3]	0.67	0.69	0.10	0.71
94 d BW to weaning[4]	0.33	0.32	0.03	0.60
WSW to weaning[1,5]	0.42	0.46	0.04	0.17
Overall[2]	0.59	0.60	0.02	0.65

[1] Weights at time of weigh-suckle-weigh were taken on a subset of calves (n=45·treatment^{-1})
[2] Calves weaned on d 217 of trial
[3] ADG calculated from gains over the 21 day period
[4] ADG calculated from gains over the 99 day period
[5] ADG calculated from gains over the 120 day period

Table 6. Effects of MFP supplementation on cow reproductive performance

	Treatments		
Item	CON	MFP	*P*-value
Cycling, %	74	77	0.69
AI conception[1], %	51	47	0.67
Overall pregnancy[2], %	78	73	0.50
AI embryonic loss[3], %	2.2	9.3	0.68
Fetal loss[4], %	1.3	5.9	0.66

[1] Cows confirmed pregnant via ultrasound at 42 d after AI, d 156 of trial

[2] Cows confirmed pregnant via ultrasound and palpation at 33 d after bulls were removed, d 217 of trial

[3] AI embryonic loss was determined on cows confirmed AI pregnant that were not pregnant at overall pregnancy check

[4] Fetal loss was determined on cows confirmed pregnant that did not have a calf the following calving season

Literature Cited

Bach, A., G. B. Huntington, and M. D. Stern. 2000. Response of nitrogen metabolism preparturient dairy cows to methionine supplementation. J. Anim. Sci. 78: 742-749.

Battaglia, F. C. 1992. New Concepts in Fetal and Placental Amino Acid Metabolism. J. Anim. Sci. 70: 3258-3263.

Beal, W. E., D. R. Notter, and R. M. Akers. 1990. Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight gain and postpartum reproduction. J. Anim. Sci. 68(4): 937-943.

Belcher, C. G. and R. R. Frahm. 1979. Productivity of two-year-old crossbred cows producing three-breed cross calves. J. Anim. Sci. 49 (5): 1195-1206.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73: 2804-2819.

Bremer, V. R., S. M. Damiana, F. A. Ireland, D. B. Faulkner, and D. J. Kesler. 2004. Optimizing the interval from PGF to timed AI in the CoSynch+CIDR and 7-11 estrus synchronization protocols for postpartum beef cows. J. Anim. Sci. 82(Suppl. 2): 106.

Brown, M. A. and A. H. Brown, Jr. 2002. Relationship of milk yield and quality to preweaning gain of calves from Angus, Brahman, and reciprocal-cross cows on different forage systems. J. Anim. Sci. 80: 2522-2527.

Chen, Z. H., G. A. Broderick, N. D. Luchini, B. K. Sloan, and E. Devillard. 2011. Effect of feeding different sources of rumen-protected methionine on milk production and N-

utilization in lactating dairy cows. J. Dairy Sci. 94: 1978-1988. doi: 10.3168/jds.2010-3578.

Chenette, C. G. and R. R. Frahm. 1981. Yield and composition of milk from various two-breed cross cows. J. Anim. Sci. 52 (3): 483-492.

Clanton, D. C. and M. E. England. 1980. Methionine hydroxy analog in supplements for lactating beef cows. J. Anim. Sci. 51(3): 539-43.

Consortium. 1988. Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching. Consortium for Developing a Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching, Am. Soc. Anim. Sci. Assoc. Headquarters, Savoy, IL.

Klopfenstein, T. 1996. Need for escape protein by grazing cattle. Anim. Feed Sci. Tech. 60: 191-199.

Krehbiel, C. R., G. D. Pulsipher, M. K. Petersen, J. E. Sawyer, and E. E. Parker. 2001. Use of urea, ruminally protected methionine, and fat alone or in combination in liquid supplements to enhance nutrient status and milk production in beef cows grazing native range. Novus International Final Report pp 1-24.

Kung, L. and L. M. Rode. 1996. Amino acid metabolism in ruminants. Anim. Feed Sci. Tech. 59: 167-172.

Lee, C., J. Oh, A. N. Hristov, K. Harvatine, M. Vazquez-Anon, and G. I. Zanton. 2015. Effect of 2-hydroxy-4-methylthio-butanoic acid on ruminal fermentation, bacterial distribution,

digestibility, and performance of lactating dairy cows. J. Dairy Sci. 98:1234-1247. doi. 10.3168/jds.2014-8904.

Meteer, W. C., D. W. Shike, and F. C. Cardoso. 2015. Prepartum and postpartum nutritional management to optimize fertility in beef cattle. Acta Scientiae Veterinariae. 43: 1286.

Ng-Kwai-Hang, K. F. and J. F. Hayes. 1982. Effects of potassium dichromate and sample storage time on fat and protein by milko-scan and on protein and casein by modified pro-milk Mk II method. J. Dairy Sci. 65: 1895-1899.

NRC 1996. Nutrient Requirement for Beef Cattle. 7th ed. National Academy Press, Washington, D.C.

Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Loor. 2013. Supplementing Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J. Dairy Sci. 96:6248-6263. doi: 10.3168/jds.2012-5790.

Patterson, H. H., D. C. Adams, T. J. Klopfenstein, R. T. Clark, and B. Teichert. 2003. Supplementation to meet metabolizable protein requirements of primiparous beef heifers: II. Pregnancy and economics. J. Anim. Sci. 81: 563-570.

Richardson, C. R. and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46: 740-745.

Rutledge, J. J., O. W. Robison, W. T. Ahlschwede, and J. E. Legates. 1971. Milk yield and its influence on 205-day weight of beef calves. J. Anim. Sci. 33(3):563-7.

Shoup, L. M., A C. Kloth, T. B. Wilson, D. Gonzalez-Pena, F. A. Ireland, S. Rodriguwz-Zas, T. L. Felix, and D. W. Shike. 2015. Prepartum supplement level and age at weaning: I.

Effects on pre- and postpartum beef cow performance and calf performance through weaning. J. Anim. Sci. 93: 4926-4935. doi: 10.2527/jas2014-8564.

Thomas, O. O. and W. J. Langford. 1978. Methionine hydroxy analog in Beef Cow Supplements given pre- and post calving. Proc. West. Sec. Amer. Soc. Anim. Sci. 29:454-455.

Varner, L. W. 1974. Supplementary diets of beef cows with methionine hydroxy analog. Montana Agr. Exp. Sta. Res. Rep. 52:34.

Waterman, R. C., C. A. Löest, W. D. Bryant, M. K. Petersen. 2007. Supplemental methionine and urea for gestating beef cows consuming low quality forage diets. J. Anim. Sci. 85(3):731-6. doi: 10.2527/jas.2006-425

Wright, J. G., W. E. Sharp. M. Compton, and R. Totusek. 1975. Supplemental Value of MHA for Range Cows. In: Oklahoma Agricultural Experiment Station Animal Science 1975 Report. Oklahoma State University. p. 22-24.